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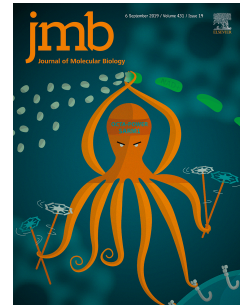
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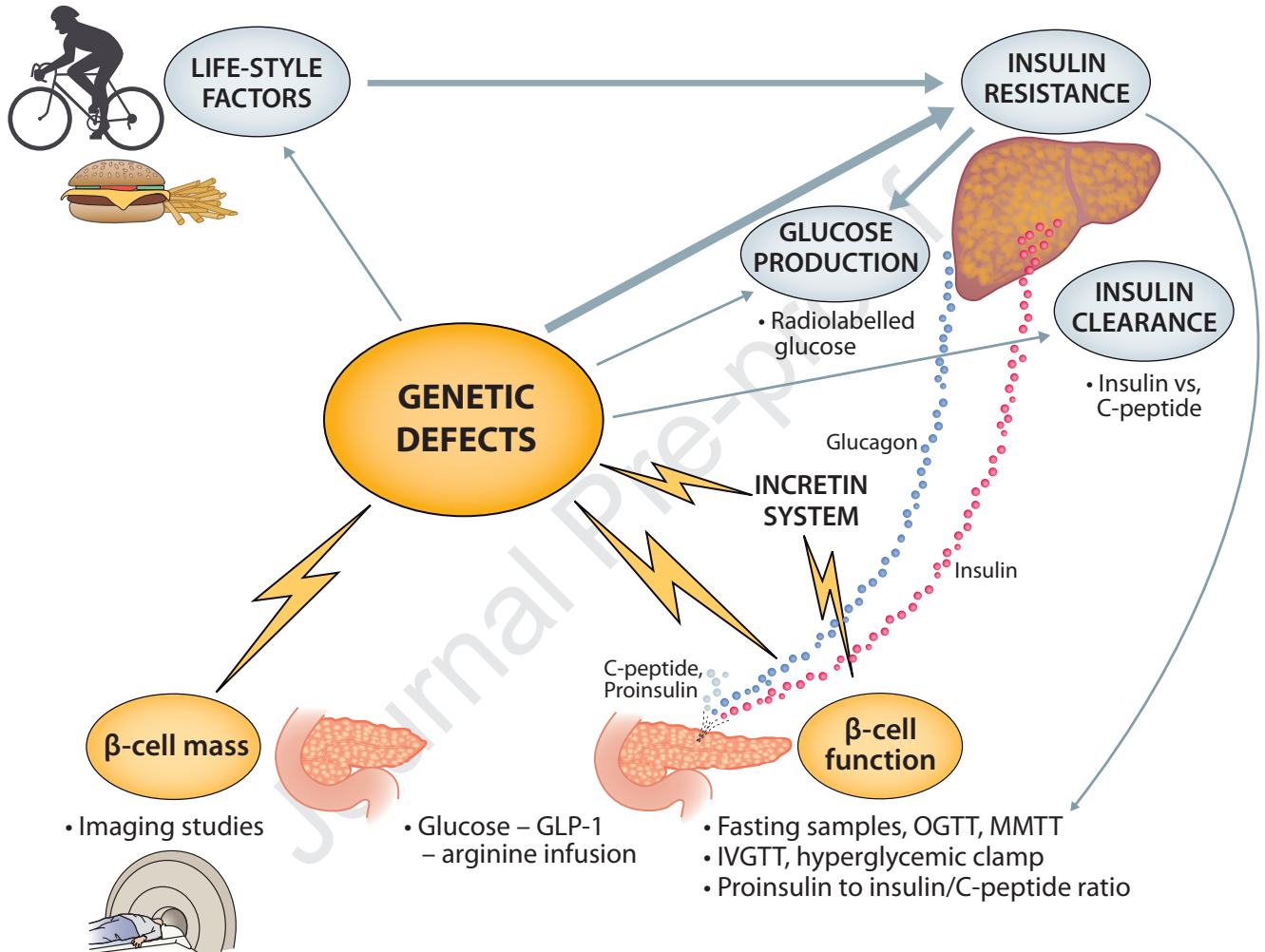
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# Human physiology of genetic defects causing beta-cell dysfunction

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## Keywords

Genetic defects; Type 2 diabetes; Human physiology; Beta-cell function

## Abbreviations

AIR	Acute insulin response
AUC	Area under the curve
CI	Confidence interval
CIR	Corrected insulin response

CNS	Central nervous system
DI	Disposition index
FPG	Fasting plasma glucose
FPIR	First-phase insulin release
GCK	Glucokinase
RFX6	Regulatory factor X, 6
HNF	Hepatocyte nuclear factor
GWAS	Genome-wide association study
GLP-1	Glucagon-like peptide-1
G6PC2	Glucose-6-phosphatase 2
GIP	Glucose-dependent insulintropic peptide (formerly, gastric inhibitory peptide)
HbA1c	Glycated hemoglobin
HOMA	Homeostasis model assessment
HOMA $\beta$	HOMA-derived index of beta-cell function
HOMA-IR	HOMA-derived index of insulin resistance
HGC	Hyperglycemic clamp
HEC	Hyperinsulinemic-euglycemic clamp
ISI	Insulin sensitivity index
IGI	Insulinogenic index
i.v.	intravenous

IVGTT	Intravenous glucose tolerance test
MODY	Maturity onset diabetes of the young
MTNR1B	Melatonin receptor 1B
MAF	Minor allele frequency
MMTT	Mixed meal tolerance test
OR	Odds ratio
OGTT	Oral glucose tolerance test
PTV	Protein-truncating variant
<i>SLC30A8</i>	Solute carrier family 30, member 8
T1D	Type 1 diabetes
T2D	Type 2 diabetes
ZnT8	Zinc transporter 8

## Abstract

The last decade has revealed hundreds of genetic variants associated with type 2 diabetes, many especially with insulin secretion. However, the evidence for their single or combined effect on beta-cell function relies mostly on genetic association of the variants or genetic risk scores with simple traits, and few have been functionally fully characterized even in cell or animal models. Translating the measured traits into human physiology is not straightforward: none of the various indices for beta-cell function or insulin sensitivity recapitulates the dynamic interplay between glucose-sensing, endogenous glucose production, insulin production and secretion, insulin clearance, insulin resistance – to name just a few factors. Since insulin sensitivity is a major determinant of physiological need of insulin, insulin secretion should be evaluated in parallel with insulin sensitivity. On the other hand, multiple physiological or pathogenic processes can either mask or unmask subtle defects in beta-cell function. Even in monogenic diabetes, a clearly pathogenic genetic variant can result in different phenotypic characteristics – or no phenotype at all. In this review, we evaluate the methods available for studying beta-cell function in humans, critically examine the evidence linking some identified variants to a specific beta-cell phenotype and highlight areas requiring further study.

## Introduction

Beta-cell dysfunction can involve different dysregulated processes including glucose sensing, response to secretory potentiators and inhibitors, proinsulin production and processing, insulin granule exocytosis etc. [1]. In humans, these cannot be studied directly *in vivo*. Instead, insulin secretion, either at fasting or after stimulus, is estimated from indices calculated from circulating peripheral concentrations of glucose and insulin or C-peptide. Most commonly insulin secretion is stimulated by oral glucose or test meal, but different intravenous (i.v.) secretagogues can also be used, including glucose, glucagon, arginine, GIP, GLP-1 or sulphonylurea (tolbutamide) [2–8]. Unfortunately i.v. sulphonylurea, GIP or GLP-1 preparations for human *in vivo* use are not available at present. Further, a graded glucose infusion has been combined with arginine boluses, sometimes potentiated with GLP1-infusion, which seems to be the best estimate of the maximal insulin secretory capacity [9].

The stimulatory tests can serve at least three purposes. Firstly, a low level of a hormone does not necessarily signify secretory insufficiency, but it can also derive from lack of demand. Hence, insulin levels of a very insulin-sensitive euglycaemic individual can be very low, while similar levels combined with high glucose reflect defective insulin secretion. Adding a stimulus for insulin secretion will bring out the difference in beta-cell function between these two alternatives.

Secondly, with stimulatory tests we can try to differentiate whether defective insulin secretion is the result of reduced beta-cell mass or defective function of beta-cells. It needs emphasizing that most tests cannot distinguish between these defects. Nonetheless, comparison of insulin responses to different secretagogues, for example sulphonylureas (SUs) and glucose, can provide indirect evidence: a sufficient response to some secretagogue but not to another signifies functionality of the defect. On the other hand, if no response is elicited by any secretagogue, the beta-cells are presumably non-existent. Pancreatic imaging is of help only in case of major structural defects. Ultrasound, computed tomography or magnetic resonance imaging (MRI) can detect total or partial pancreatic aplasia, as well as signs of marked acute or chronic pancreatitis, whereas

functional imaging with positron emission tomography employing a radiolabeled GLP1-analogue, single-photon emission computed tomography, and cation-enhanced MRI allow focusing on pancreatic islets [10,11]. However, even these methods are incomplete regarding sensitivity/specificity, spatial resolution or tissue penetration of the radioligand, and none of the imaging modalities specifically detects beta-cell mass [12,13]. Nor is the detection of beta-cell loss possible *in vivo*, as pancreatic biopsies are not available due to complications associated with the procedure. Even post-mortem studies are few [14,15].

To detect subtle defects in beta-cell mass or function, a secretory stimulus combined with blood sampling at multiple time-points may be required. For minute effects, a combination of different stimuli (e.g. glucose and arginine) may be necessary to tease out a difference. Another possibility to unmask subtle changes in beta cell function would be to increase the need of insulin. Obviously, we cannot modify the degree of insulin resistance in humans with high-fat diet, restriction of movement or other methods applied in animal studies. A natural increase in insulin resistance during pregnancy could provide a powerful setup for physiological studies on variants affecting beta-cell dysfunction, if large enough cohorts with gestational diabetes were available. In line with this, monogenic diabetes is often exposed during pregnancy [16].

Thirdly, the use of different stimuli can reveal qualitative differences in beta-cell function. Thus the choice of a stimulus depends on whether the interest lies in glucose-responsiveness, incretin response or other processes. Briefly, glucose stimulates insulin secretion through the  $K_{ATP}$  channel closure and membrane depolarization. The insulinotropic incretins, GIP and GLP-1, act directly on GIP receptor and GLP-1 receptor and potentiate glucose-mediated insulin secretion by increasing intracellular cAMP levels and thus the  $K_{ATP}$  channel activity [17]. Also glucagon, binding to glucagon receptors and GLP-1 receptors, elicits glucose-mediated insulin secretion [4]. On the other hand, sulphonylureas directly close the  $K_{ATP}$  channel independently of glucose; genetic



variation in the  $K_{ATP}$  channel can modify the response to sulphonylureas. However, arginine has an effect independent of both glucose and the  $K_{ATP}$  channel. Being a positively charged amino acid, it is transported by cationic amino acid channels into to beta cell, leading to electronic depolarization [4].

Functional characterization of a genetic variant, which is presumed to affect beta-cell function, usually involves engineering the mutation in beta-cell lines, induced pluripotent stem cell lines differentiated into beta-cells, or global/tissue-specific knock-out or knock-in animal models. These models have a controlled genetic background, which allows of testing the specific effect of a single variant. On the contrary, in humans, besides the variant of interest also all other variation in the genome and epigenome modulates the studied trait – together with the effects of life-style. Co-occurring variants can also have opposite effects on the trait, which can mask the true effect of the variant of interest, as we recently showed for variants in *SLC30A8* [18]. Another example is the common p.Val219Leu variant in *G6PC2*, which is associated with reduced G6PC2 protein expression and reduced fasting plasma glucose *in vitro*, but paradoxically with higher glucose at a population-level [19]. The rationale is that the glucose-lowering coding allele is restricted to a haplotype that carries the glucose-raising allele of another regulatory variant, which masks the true functional direction of the effect of p.Val219Leu [19]. These examples highlight the need for genetically matched control groups beyond ethnicity. Therefore, to characterize the human phenotype of variants in *HNF1A*, *RFX6* and *SLC30A8* described below, we employed a recall-by-genotype setting involving cascade-testing to increase the number of carriers (stepwise recruitment of first-degree relatives of mutation carriers along as new carriers were identified) and recruitment of family-based controls, which enabled us to control for both background genetics and partially also for environmental and life-style factors.

## How to assess insulin secretion *in vivo*?

### Caveats to consider

Most studies rely on measurement of glucose and insulin in the blood circulation. However, the relationship between systemic concentrations of insulin and glucose is affected by many factors: glucose-sensing, secretory potentiators and inhibitors of insulin secretion, insulin-secretory capacity, glucagon secretion and endogenous glucose production, glucose uptake by the tissues, glucose effectiveness, glucosuria (reviewed in [3]). Furthermore, as the liver clears most of the secreted insulin during the first-pass transit from the pancreas, the systemic insulin concentration does not reflect total insulin secretion. Hence, C-peptide, secreted from beta cells in equimolar amount with insulin but with negligible hepatic clearance, is often measured instead. One should bear in mind that, C-peptide is sensitive to sample handling as it is quickly destroyed by proteases in fresh blood samples, and its long biological half-life (compared with insulin) precludes studying quick dynamic changes. The best option is to measure both insulin and C-peptide, which also allows estimating hepatic insulin clearance pending an adequate series of consecutive blood samples.

One should also keep in mind that assessing insulin secretion in individuals with marked hyperglycaemia is unreliable as chronic hyperglycaemia inhibits insulin secretion [20]. Hence, the tests are usually restricted to those without overt hyperglycaemia (e.g. fasting glucose <10 mmol/l), which causes bias in the studies.

A renewed interest has focused on proinsulin concentrations and proinsulin-to-insulin ratio, which has been considered an indicator of beta-cell stress or even a marker of the maximal beta-cell secretory capacity [21,22]. However, most of the original work on this ratio relied on radioimmunoassays employing polyclonal antibodies with major cross-reactivity. Thus, the

proinsulin assay detected both intact proinsulin and conversion intermediates (different des- and split-proinsulins), and the insulin assay detected also proinsulin and its conversion intermediates [21]. Back then, the ratio at least partly served as a way to obtain “clean” insulin concentrations. It is unclear how the ratios obtained with current, specific monoclonal assays correlate with the previous studies. In the same way, the old C-peptide assays also detected proinsulin.

It takes three hours for 90% of the newly synthesized proinsulin to be converted to insulin in the beta-cell granules, and under stimulated conditions of elevated glucose as much as 50% of the newly synthesized (pro)insulin can be released within 3 hours [23]. Thus, both a primary impairment in proinsulin processing and an increased secretory demand on the beta cells could lead to an increased proinsulin-to-insulin ratio. Unfortunately, these mechanisms cannot be studied *in vivo* so we need to rely on the ratio. In this case, substituting insulin with C-peptide for the ratio might be beneficial as C-peptide escapes the clearance in the liver.

### **Fasting samples, limited but straightforward view on human physiology**

For practical reasons, large studies often rely on fasting blood samples to estimate beta-cell function. Indices derived from fasting plasma glucose and plasma/serum insulin or C-peptide provide a robust estimate of insulin secretion and sensitivity. HOMA $\beta$  is generally used for insulin secretion and HOMA-IR for insulin resistance ([24,25], available at

[www.dtu.ox.ac.uk/homacalculator](http://www.dtu.ox.ac.uk/homacalculator), reviewed in [3,26]). The original computer model of

HOMA[24,27] is often substituted with less accurate simple approximations (HOMA $\beta$   $\frac{20 \times \text{insulin}}{\text{glucose} - 3.5}$

and HOMA-IR  $\frac{\text{glucose} \times \text{insulin}}{22.5}$ , where concentration of glucose is given in *mmol/l* and insulin in

$\mu\text{U/mL}$ ). Although the performance of these indices tends to be evaluated based on how well they correlate with results from glucose tolerance tests or clamp methods, one should keep in mind that fasting concentrations and those measured in response to a stimulus represent different aspects of beta-cell function and human physiology.

It is essential to assess insulin secretion together with insulin sensitivity [26]. Although a normoglycemic insulin-sensitive individual has no physiological need to boost insulin secretion, the HOMA $\beta$  index could suggest impaired beta cell function if assessed without HOMA-IR (especially in individuals having both a low HOMA $\beta$  and low HOMA-IR, (*Figure 1*). On the other hand, in the context of normoglycaemia, mildly impaired insulin secretion could remain unexposed due to high insulin sensitivity. Further, these simple indices do not distinguish between different etiological mechanisms of hyperglycemia: an individual with life-long stable fasting hyperglycemia as a result of a *GCK* defect can score identically with a patient presenting with an early type 1 diabetes, as they both demonstrate fasting hyperglycemia and low or normal insulin concentrations. This is exemplified by a GAD-antibody-positive 31-year-old man presenting with HOMA $\beta$  of 26.4% (model)/16.6% (simple formula) and HOMAIR of 0.58/1.15 (fasting plasma glucose 7.6 mmol/l and insulin 3.4 mU/l) who later progressed to insulin-deficient T1D (C-peptide <0.01 nmol/l), whereas a 44-year-old man with similar values (HOMA $\beta$  26.5%/15.7%, HOMAIR 0.56/1.10, glucose 7.5 mmol/l, insulin 3.3 mU/l), was diagnosed with GCK-MODY (Kettunen J, unpublished data from the Botnia Study). Obviously, the larger the study, the less these caveats affect the results, but one should be cautious when using these indices for individual assessment.

### Orally stimulated insulin secretion

Compensatory mechanisms can maintain fasting normoglycemia despite modest disturbances in glucose metabolism, which can be unmasked with tolerance tests. The most commonly used – and the only universally standardized test - is the oral glucose tolerance test (OGTT), which is used for diagnosing diabetes and gestational diabetes. It involves a standard glucose dose (usually 75 g in adults) ingested in 5 minutes, and blood sampling at fasting and at 120 minutes for plasma glucose [28,29]. Additional sampling at 30 minutes or at other time points, complemented with measurements of serum insulin or C-peptide, allows calculation of various indices of insulin

secretion and insulin sensitivity. The most commonly used are insulinogenic index (IGI,  $\frac{\Delta\text{insulin}}{\Delta\text{glucose}} = \frac{\text{insulin(at } 30') - \text{insulin(at fasting)}}{\text{glucose(at } 30') - \text{glucose(at fasting)}}$ ) [30], 30-minute values occasionally substituted by 60-minute values)

and corrected insulin response (CIR<sub>30</sub> or CIR<sub>120</sub>,

$$\frac{\text{insulin}[\mu\text{U/mL}](\text{response, usually at } 30'/120') - 100}{\text{glucose}[\text{mg/dl}](\text{response, usually at } 30'/120') - 70} = \frac{\text{insulin}[\mu\text{U/mL}](\text{response, usually at } 30'/120') - 100}{18 \times \text{glucose}[\text{mmol/mol}](\text{response, usually at } 30'/120') - 70} \quad [31]) \text{ for}$$

insulin secretion, and the composite insulin sensitivity index (ISI or Matsuda index) for insulin sensitivity [32]. A disposition index (DI) can aid in relating secretion to sensitivity [3]. This is exemplified by a longitudinal analysis of a population study, where during the follow-up the insulin sensitivity decreased and, although CIR seemed to increase accordingly, the DI (CIR\*ISI) actually decreased in those with T2D risk genotypes [33]. However, the equation for DI has not been standardized [3]. CIR or IGI at 30 minutes are considered surrogate measures of first-phase insulin secretion, although also the second-phase secretion already contributes at this stage [3]. During a tolerance test, glucose and insulin area under the curve (AUC) can serve as a proxy for the total glucose or insulin response. In addition, model-based indices provide more accurate estimates of glucose metabolism but they require sampling at multiple time-points (preferably at least seven) or time-points not usually included (like 90 minutes) [34–36].

Figure 2 shows a comparison of OGTT-derived indices with fasting indices for insulin secretion and sensitivity. HOMA-IR correlates (inversely) rather well with the OGTT-derived insulin sensitivity index (ISI) in both diabetic and non-diabetic individuals, whereas the relationship between HOMA $\beta$  and either corrected insulin response (CIR<sub>30</sub>) or C-peptide response at 2 hours during the OGTT varies considerably, underlining the biological difference between fasting and stimulated insulin secretion.

A mixed meal tolerance test [37–39] (MMTT) is an alternative to an OGTT in a research setting, although it's not being standardized hampers comparison of studies. Differing from OGTT, the mixed meal contains various carbohydrates but also protein and fat, which influence the insulin secretory response and glucose excursion and potentiate the incretin response. The same indices

for glucose and insulin secretion and insulin sensitivity can be applied to MMT as described for OGTT.

Oral tests elicit physiological secretion of incretin hormones GLP-1 and GIP from enteroendocrine cells (reviewed in [6,17,40]). Incretins potentiate the insulin secretory response stimulated by glucose and amino acids resulting in a higher insulin secretion after an oral glucose stimulus compared with an i.v. glucose stimulus, which is called the incretin effect. The magnitude of the physiological incretin effect can be tested with an isoglycaemic clamp: the individual's glucose response during an OGTT is replicated with an i.v. glucose infusion (resulting in exactly the same glucose levels as during the OGTT), and insulin response is measured. A difference in insulin response during an OGTT or MMTT can result from defective incretin secretion or defective beta-cell response to incretins as well as from a defective beta-cell response to glucose or amino acids. However, also differences in glucagon response to incretins, gastric emptying or intestinal absorption could play a role. The magnitude of the incretin secretory response can be estimated from serum/plasma samples, preferably collected in tubes containing dipeptidyl peptidase-4 (DPP4) inhibitors [41]. However, the circulating concentrations of the hormones do not necessarily reflect the magnitude of the incretin effect on insulin-secretion, which is likely to be neurally mediated rather than a direct effect on GLP-1 or GIP receptors on the beta-cells [40]. The hormones are also rapidly cleared from the circulation and only a fraction reaches the pancreas. In any case, some genetic variants are known to affect circulating incretin concentrations [42–44]. The beta-cell response to incretins can be tested with administration of i.v. GIP or GLP-1.

### **Intravenous stimulants of insulin secretion and action**

Although uncommon these days, i.v. glucagon bolus (1 mg) has also been used to promote insulin secretion mostly combined with a single measurement of C-peptide at 6 minutes, which has

substituted serial sampling [45–47]. Glucagon test is a robust method to assess insulin secretion capacity without the incretin effect, but typical adverse effects include nausea [3,4].

I.v. glucose administration stimulates the beta-cells directly without an accompanying incretin effect. The most commonly used test involves an i.v. glucose bolus (0.3 g/kg body weight) and blood sample collection after the bolus at every 1-2 minutes during the first 10 minutes, and every 5-10 minutes thereafter (intravenous glucose tolerance test, IVGTT). The sum of insulin values at 1 and 3 minutes or the incremental insulin response during the first 10 minutes is considered to represent the first-phase insulin response, and the incremental insulin response from 20 min to 60 min the second-phase insulin response. “Bergman’s minimal model” allows for estimation of insulin sensitivity ( $S_i$ ) and glucose effectiveness ( $S_G$ ) through repeated sampling after the i.v. glucose bolus [48], often with an additional bolus of insulin or sulphonylurea 20 min after the glucose bolus.  $S_G$  is an insulin-independent measure for glucose to lower its own concentration, and  $S_i$  an insulin-dependent measure of glucose disappearance; they differentiate the physiological actions of both glucose and insulin to suppress endogenous glucose production and to increase glucose uptake in tissues. Some limitations and undermodelling of the minimal model can be overcome by replacing insulin measurements with C-peptide or using both combined (discussed in [36]). As an alternative to the minimal model, we have employed a combination of an IVGTT followed by a euglycaemic clamp (see below) during the same day, which provides estimates of both insulin secretion and insulin sensitivity (the Botnia clamp [49]).

### **Refined phenotypes obtained with intravenous clamp studies**

A more powerful way to bring out differences in insulin secretion is a hyperglycemic clamp (HGC), which involves generating constant hyperglycemia (for example, 10 mmol/l [9]) through a continuous glucose infusion [9,50]. The rate of the infusion is determined by the insulin secretion rate (estimated from serum insulin concentration), insulin clearance (estimated from serum insulin and C-peptide concentration) and glucose uptake in tissues. However, the HGC can be criticized

for predisposing beta cells to unphysiological stress, and the immediate first-phase insulin response cannot be assessed by it. When studying patients with genetic variants that alter glucose sensing, like in GCK-MODY, measurement of insulin secretion at gradually increasing glycemic levels (a graded glucose infusion) [51,52], could provide valuable information on both glucose responsiveness and, if combined with arginine bolus at highest glucose, also maximal insulin secretion [2,9]. An even more powerful method to measure maximal insulin secretion capacity is combination of hyperglycaemia, GLP1-infusion and arginine boluses.

A hyperinsulinemic euglycemic clamp (HEC)[50,53] is considered the gold standard to assess insulin sensitivity *in vivo*. A continuous insulin infusion raises serum insulin to a target level while a compensatory dynamically adjusted glucose infusion keeps plasma glucose constant (clamped), usually at 5.5–6.5 mmol/l. The glucose infusion rate in the steady state defines glucose uptake as a measure of insulin sensitivity. A variation with labelled glucose infusion (deuterium, tritium) provides an estimation of endogenous glucose production and, thereby, differentiating between hepatic and peripheral insulin sensitivity. When studying patients with genetic variants that alter glucose sensing, modifications of the glycaemic targets should be considered. For example, the routine clamping target of a HEC of 5.5 or 6.5 mmol/l is inappropriately low for some *GCK* gene defects [52,54].

The use of time- and resource-consuming clamp studies have usually been restricted to small studies or subcohorts focusing on clear disturbances in insulin secretion or insulin sensitivity. A mitochondrial DNA variant (m.3243A>G, causing mitochondrial diabetes) was reportedly associated with defective insulin secretion (by HGC, IVGTT, and arginine challenge) and decreased peripheral insulin sensitivity (by HEC) [55,56]. Monogenic *HNF1A* defects (resulting in *HNF1A*-MODY) are associated with reduced insulin secretion irrespective of study method (fasting samples, OGTT, IVGTT, HGC), while evidence on insulin sensitivity is somewhat contradictory [57–59]. Patients with *HNF1B* defects have reduced hepatic but normal peripheral insulin sensitivity [59]. An interesting approach was employed in a hypoglycaemic hyperinsulinemic clamp



study showing that in individuals with GCK-MODY, decrease of glucose concentration lead to an earlier decline in insulin secretion (C-peptide) and increase in glucagon and epinephrine secretion, as compared to individuals with type 2 diabetes (matched for fasting glycemia) and controls [52]. This suggests that the alpha-cell and the hypothalamic glucose sensing as well as the threshold for counterregulatory response is also impaired.

Outside gene defects with manifest association with diabetes, research on less severe defects often require larger sample populations. A study on mechanistic associations of T2D-associated variants (*JAZF1*, *CDC123/CAMK1D*, *TSPAN8/LGR5*, *THADA*, *ADAMTS9*, *NOTCH2/ADAMS30*, *DCD*, *VEGFA*, *BCL11A*, *HNF1B*, *WFS1*, and *MTNR1B*) in 336 participants had enough power to show an effect on insulin response during the HGC only for *CDC123/CAMK1D*, *ADAMTS9*, *BCL11A*, and *MTNR1B* ( $P < 6.9 \times 10^{-3}$ ) [60], while another study in 146 participants showed an association with a significant reduction in first-phase insulin secretion for *CDKAL1* and *IGFBP2* [61]. Genetic studies utilizing an IVGTT include a GWAS study in 5,567 participants to characterize type 2 diabetes variants and their associated first-phase insulin secretion [62].

## What can monogenic diabetes teach us about genotype-phenotype correlation?

Before analyzing the evidence for beta cell dysfunction associated with the T2D gene variants, let us consider the human phenotype of the established monogenic variants associated with beta-cell dysfunction. *Maturity-onset diabetes of the young* (MODY) was initially a clinical diagnosis of early-onset non-insulin dependent diabetes with a dominant pattern of inheritance [63,64], which largely has guided patient selection for genetic screening. Thus, most patients identified with a monogenic variant in *HNF1A* or *HNF4A* in the first wave used to have a typical MODY phenotype, including a defective insulin response to glucose and progression to diabetes at young age. However, the phenotypic associations have become less clear, when larger groups of either non-diabetic individuals or diabetic patients without an *a priori* clinical suspicion of MODY have been sequenced

for the MODY genes (see below). For example, despite conclusive *in vitro* data on the beta-cell effects of pathogenic *HNF1A* variants, the variation in the human phenotype of HNF1A-MODY remains puzzling. Why do some individuals with pathogenic variants in *HNF1A* develop diabetes at age 10, while others with the same variants might remain free of diabetes long after the age of 50?

Nation-wide population studies provide a powerful tool to avoid ascertainment bias. A recent analysis of unselected data from the UK biobank revealed that less than 10 % of the heterozygous carriers of the *HNF4A* p.Arg114Trp were diabetic by the age of 40 years, which contradicts the data from MODY cohorts [65]. Another, partially population-based study in 2013 (with less conservative variant curation) found no association between pathogenic variants and diabetes [66]. However, while population-based studies potentially reduce ascertainment bias, they often lack systematic and repeated assessment of glycemic status. Without such systematic assessment, even monogenic diabetes might go unnoticed for years or decades, as patients with absent-to-mild symptoms hardly seek medical attention. Moreover, in some MODY subtypes, including HNF1A-MODY and HNF4A-MODY, marked hyperglycemia might only occur after a stimulus (e.g. in response to carbohydrate load in OGTT or every-day meal) [51].

Accordingly, among our families with the most common HNF1A-MODY variant (p.Gly292fs), the majority of the variant carriers had a diabetic value at 2 hours (65 patients), but only half of them had a diabetic fasting value (36 of 69 patients) during their first diabetic OGTT. HbA1c performed as poorly as fasting plasma glucose, as only 48% had a diabetic value (Unpublished data). Thus, diagnosing or excluding early diabetes in a carrier of a pathogenic *HNF1A* variant without an OGTT (or other test to stimulate insulin secretion) has the sensitivity of flipping a coin. Similarly, if studies solely depend on self-reported diabetes or diagnostic codes in hospital records, the sensitivity to estimate prevalent or incident diabetes is compromised [67–70]. *Figure 3* illustrates the influence of awareness and early screening on timing of the diagnosis of diabetes in HNF1A-MODY.

An important but often neglected aspect to consider is the right time window for characterization of human physiology of the genetic defects as the pathophysiology is often age- or time-dependent. While most patients with HNF1A-MODY display a typical glucose and insulin response during OGTT (shown in the upper panel of *Figure 4A*), other carriers with exactly the same *HNF1A* variant can have quite normal insulin and glucose response (*Figure 4A*, lower panel). In some cases, even repeated testing well into adulthood fails to show typical phenotypic characteristics (data not shown). No obvious explanation springs up but the degree of glucosuria during the test, which is rarely measured, could play a role. Likewise, *figure 4B* shows variation in the OGTT response of *GCK* variant carriers (middle and lower panels of *figure 4B*). The variation in the human phenotype of the established monogenic beta-cell dysfunction genes forebodes problems for deciphering the phenotype of the common variants.

### **T2D or low-penetrant MODY – the dilemma of *RFX6* variants**

These phenotyping problems were evident in our study of protein-truncating variants (PTV's) in *RFX6* associated with what has been considered low-penetrant MODY [42]. Yet, for unknown reasons, PTVs in *RFX6* appeared to lead to diabetes only in individual patients, whereas many carriers showed no obvious beta cell dysfunction. In this case, the difference between rare high-risk T2D variants and variants causing low-penetrant monogenic diabetes is far from clear. One might even question, whether the dichotomous definition of having or not having diabetes is relevant to investigating these variants. Instead, maybe we should use comparative analyses of continuous glycemic or insulin secretion trait variables to evaluate the “diabetic potency” of a gene defect.

Although neonatal diabetes caused by biallelic *RFX6* defects results from disturbed pancreatic development [71,72], the mechanism of MODY associated with monoallelic *RFX6* defects might involve other mechanisms. Although *RFX6* directly regulates insulin production in beta cells [72], it

also increases GIP secretion from intestinal K cells [73]. Indeed, the PTV carriers had lower fasting and stimulated serum GIP concentrations compared to control individuals. Thus, PTVs in *RFX6* could contribute to diabetes also indirectly via GIP-mediated effect on islet-function through eliciting insulin secretion from beta cells and glucagon secretion from alpha cells as well as through promoting beta cell survival. To date, the indirect effects on pancreatic function e.g. via the incretin or nerve system have not really been studied with respect to any genetic variants considered to affect beta-cell function [74].

## Common variants associated with beta-cell function

A genetically programmed failure of the beta cell to compensate for insulin resistance is considered the main culprit in T2D [75]. Accordingly, of the more than 400 genetic variants associated with T2D in GWAS studies, a large proportion appears to influence insulin secretion [76–78]. However, considering the minimal individual effect of the variants on diabetes risk or insulin secretion, very large cohorts are required to prove an association. Hence, most of the evidence for beta-cell dysfunction inevitably relies on association of the variants with rather simple phenotypic traits based on fasting measurements of glucose and insulin, sometimes complemented with C-peptide or proinsulin [22]. The caveats discussed above apply, measures of insulin secretion are rarely adjusted for insulin sensitivity even in non-diabetic cohorts and stimulatory tests tend to be available only for subgroups. Thus, it is difficult to make the step from an association between a variant and plasma concentration of glucose and insulin, or indices calculated from them, to the interpretation that the variant would be associated with decreased insulin secretion and not increased insulin sensitivity. Recent approaches applying soft clustering on a number of traits only partly circumvent these problems [77,79].

Instead of reviewing data for all variants associated with insulin secretion in different studies (for detailed trait associations, see [22]), we concentrate on the variants Mahajan *et al.* [77] found to affect insulin secretion through clustering analysis in a meta-analysis. Twenty-one variants were associated with insulin secretion (Supplementary figure 6B in [77]; eight also with high proinsulin and 21 with low proinsulin), whereas another 35 variants were considered likely to have an impact on both insulin secretion and insulin action. Based on the results reported by Mahajan for different traits [77], we set out to re-explore the clinical evidence for an insulin-secretory defect. In this exercise, we included the 25 variants with data available for corrected insulin response adjusted for insulin sensitivity during an OGTT (CIR\*ISI, N~5000), HOMA $\beta$  (N~46,000), 2-hour glucose (N~50,000) and proinsulin (N~10,000), and either  $P < 1 \times 10^{-4}$  for any of the traits, or nominally significant ( $P < 0.05$ ) association in case of CIR\*ISI or proinsulin (Table 1, Supplementary table 11 in [77]). Note that data for different traits might have come from different cohorts in the meta-analysis, and the same individuals were not necessarily tested for all included traits.

Considering either low CIR\*ISI or a combination of low HOMA $\beta$  and high 2-hour glucose as strong evidence of decreased insulin response to glucose (2-hour insulin was not available), a significant association was considered for nine of the “insulin secretion” variants in (or near) *MTNR1B*, *CDKAL1*, *HHEX-IDE*, *CDKN2A-B*, *SLC30A8*, *TCF7L2*, *ANK1*, and *GCK* (Table 1). Obviously, a similar phenotype could also result from increased hepatic clearance, as shown for another *CDKAL1* variant with an association with insulin clearance [80]. Another three variants were strongly associated with 2-hour glucose and modestly with HOMA $\beta$  (*ADCY5*, *IGF2BP2*) or vice versa (*GLIS3*) - but not with measures of insulin sensitivity - and thereby considered to imply “probable insulin secretory defect”. Regarding the variants associated only with 2-hour glucose, we considered the association possible evidence for defective insulin response to glucose (“possible insulin secretory defect”), as the variants were not associated with insulin resistance (estimated with HOMA-IR or ISI,  $P > 0.05$ , Supplementary table 11 in [77]). Independent data for corroboration of these findings are challenging to find, because most large cohorts have been included in these meta-analyses in the first place. It also needs to be pointed out that some quite established beta-

cell genes like *KCNJ11* were not included in this analysis. Obviously, the rest of the variants included in the clusters could still be associated with clinically significant insulin secretory defects in humans, but more *in vivo* data would be needed to prove it. Whether we can upgrade association to causality regarding these variants and beta-cell function, depends on functional studies (beyond the scope of this review).

The available data do not allow for differentiating defective glucose-sensing from incapacity to synthesize, process or secrete proinsulin/insulin (including paucity of beta-cells). No direct association could be shown with single variants and proinsulin concentration in the subcohort of 10 000 individuals with proinsulin data (except perhaps for rs7903146 [*TCF7L2*];  $P=0.00464$ , and rs1359790 [*SPRY2*];  $P=0.0042$ ; *Table 1*). However, the genetic variants associated with T2D and insulin secretion have been reported to fall in two seemingly different clusters, one associated with low, and the other with high proinsulin, suggesting mechanistic differences behind insulin deficiency [77,79,81]. Udler *et al.* showed that genetic risk scores (GRS) based on the clusters were associated with decreased or increased proinsulin ( $P<10^{-17}$ ) [79]. Four of the variants included in the GRS (in/near *TCF7L2*, *SLC30A8*, *C2CD4A-B*, *CENTD2* a.k.a. *ARAP1/STARD10*) have previously shown significant association with fasting proinsulin concentration adjusted for insulin [81]. Regarding the *TCF7L2*, *SLC30A8* and *C2CD4A-B* variants, the proinsulin-raising allele was seen together with higher 32,33-split proinsulin, lower fasting insulin, higher fasting glucose and lower IGI during the OGTT - originally interpreted to suggest dysfunction in the early steps of insulin synthesis. However, in case of the variant in *SLC30A8* (rs11558471, which is in linkage disequilibrium with rs13266634 discussed below), it seems that in fact the major allele (with a C at rs13266634, referred to as Trp325 below) actually enhances proinsulin processing thus decreasing proinsulin-insulin and proinsulin-C-peptide ratio [18]. Interestingly, an association was also seen with *PCSK1*, which encodes the prohormone convertase.

### ***SLC30A8* – The power of family-based studies**

The common missense variant (rs13266634, c.973T>C, p.Trp325Arg) in the *SLC30A8* gene, encoding the Zinc transporter 8 (ZnT8), was among the first variants strongly associated with T2D risk with an OR of 1.18 for the arginine allele [82]. However, since then, several studies and meta-analyses have verified that actually the minor allele at this position lowers T2D risk, fasting glucose ( $P=1.63 \times 10^{-46}$ ) and HbA1c ( $P=8.5 \times 10^{-28}$ ) [77]. Moreover, *SLC30A8* harbours more than 30 low-frequency loss-of-function (LoF) or missense variants conferring protection against T2D [83]. Indeed, based on their series of protective alleles, Flannick *et al.* [83] argued that decreased T2D risk was the typical effect of *SLC30A8* missense variation and not unique to haploinsufficiency, as was first presumed when the p.Arg138\* (rs200185429, c.412C>T) and p.Lys34Serfs\*50 (rs587777582, c.101\_107del) were found to confer 53%-80% protection against T2D [84]. Although the encoded protein, ZnT8, is directly connected to insulin secretion, being responsible for insulin transport to the secretory vesicles and hexamer formation, the mechanism behind the protection remained unclear for a long time – much due to the conflicting results from animal and *in vitro* studies. It took us five years to recruit enough LoF carriers and related non-carriers through cascade screening and to complete a detailed metabolic testing to prove the physiological consequences of the p.Arg138\* variant [18]. In the end, we could show that rare p.Arg138\*, and to a lesser extent major common variant allele (Trp325) were associated with a higher insulin secretory response during OGTT and test meal (Trp325 also during an IVGTT), whereas the carriers had no differences in glucagon, GLP-1 or free fatty acid response compared with family- or population-based controls [18]. Lower proinsulin/C-peptide and proinsulin/insulin ratios suggested effects on proinsulin conversion as a mechanistic explanation in addition to an enhanced insulin responsiveness to glucose.

However, the story may be more complex. Circulating zinc levels have also been associated with diabetes (reviewed in [85]). Moreover, a report in 1796 individuals (785 T2D patients) from China showed that an inverse association of plasma zinc concentration with T2D was modified by the p.Trp325Arg genotype [86], but to our knowledge, no one has tried to replicate that finding. We

found no clear correlation between circulating zinc concentration and insulin secretion or presence of p.Arg138\* or p.Trp325Arg in a small subset of participants who had data for zinc concentrations [18]. Unfortunately, retrospective analysis in larger cohorts from stored samples is not possible as the measurement of zinc or other trace elements requires that samples be collected in special tubes, and the zinc concentration must be adjusted for copper and albumin concentration. Considering the potential of the ZnT8 for pharmacological intervention, studies on the effect of zinc consumption are called for.

### Controversies around *MTNR1B*

Melatonin is synthesized from serotonin in a circadian manner mainly in the pineal gland with the highest circulating levels at night and low values during the day. The synthesis and direct secretion is under the control of the central biological clock, which, in turn, is regulated through feed-back mechanism by the rhythmic melatonin pulses, which also serve to influence the circadian regulation of peripheral tissues [87]. Circulating levels of melatonin peak at ~200 pg/ml in the middle of the night and drop to ~10 pg/ml during day time; the half-life of the hormone in the circulation is less than 20 min. Melatonin has a direct inhibitory effect on insulin release but also a stimulatory effect on glucagon secretion (reviewed in [88]).

A non-coding variant rs10830963 in the *MTNR1B* gene, encoding the melatonin receptor 1B, has shown consistent association with the risk of T2D, increased fasting glucose and HbA1c (but not 2-hour glucose during OGTT) as well as with decreased early insulin response to glucose in several studies [77,89–91]. Each G allele of the variant increases the risk of T2D by OR of 1.09 (95% CI 1.05;1.12) and fasting plasma glucose by 0.07 mmol/l (95% CI 0.06;0.08) [91]. Similarly, a consistent allele dose-dependent decrease in early insulin secretion has been shown in non-diabetic individuals from several cross-sectional studies: OGTT-based measurements in the Finnish Botnia, PPP-Botnia, METSIM and Helsinki Birth Cohort studies (N=11,601, CIR) [90], and



the Danish Inter99 Study [91] (N=5553, insulinogenic index), and an impaired insulin response during IVGTT (FPIR, AIR) in the Botnia (N=505), FUSION (N=522) and Inter99 (N=5553) studies. Also, longitudinally, each G allele was associated with an increase in FPG and with a decrease in CIR and DI between baseline and follow-up in the Botnia Prospective and PPP-Botnia studies but not with changes in HOMA-IR or ISI [90].

While the association of the risk variant with increased fasting glucose and decreased insulin secretion is well established, the suggested effect on insulin-sensitivity is less clear. An association with decreased insulin sensitivity (ISI, beta -0.04, SE 0.012,  $P=6.3 \times 10^{-4}$ ) was seen in non-diabetic individuals from the population-based PPP-Botnia study (N=4654) at baseline [92]. The effect was roughly of the same size, but statistically insignificant in the subgroup with follow-up OGTT data (N=3422; ISI at baseline/follow-up: -0.047 (95%CI-0.093; 0.000) / -0.032 (-0.081; 0.017) [93]. On the other hand, Sparsø *et al.*[91] found no association with fasting or OGTT-based measures of insulin sensitivity (HOMA-IR, BIGTT-Si) in the Inter99 Study, nor with whole-body insulin sensitivity measured with euglycaemic hyperinsulinemic clamp in a small group of elderly individuals (N=77). However, they reported an association with decreased hepatic insulin sensitivity in the latter group. Further, a recent meta-analysis of GWAS data found no association with either HOMA-IR (N=46186) or ISI (N=5318) [77]. The effect, if there is one, is in any case smaller than that on insulin secretion. Such a small effect would be difficult to see unless the genotype groups were well matched, as many confounding factors predominantly affect insulin sensitivity (BMI, exercise, quality of sleep, other stress factors). Of note, no association has been observed with the risk variant and quality of sleep [93,94]. One confounding factor could be seasonal variation in day-light length, which has been reported to interact with the association between the variant and metabolic parameters [93,95], although more studies are needed to prove this.

Outside the central nervous system (CNS) and retina [90,96], *MTNR1B* is predominantly expressed in the pancreatic beta-cells [96]. A mechanistic explanation linking the clinical phenotype to the non-coding variant came from studies showing that the rs10830963 risk variant

leads to increased expression of the receptor in beta-cells and increased signaling through the receptor, which in turn was associated with lower insulin-secretion [90,94]. In line with this, an intervention with a daily dose 4 mg of melatonin for three months led to a decrease in insulin secretion and an increase in glucose level in all individuals but particularly in the risk genotype carriers in a small recall-by-genotype-based study (N=23 with GG, 22 with CC genotype) nested in the PPP-Botnia Study [94]. Similar results regarding inhibitory effect on insulin secretion, were obtained in an acute dosing (5 mg) study on 17 (11 CG, 6 CC) individuals [97]. However, these results are difficult to combine with data on rare LoF variants leading to decreased receptor signaling, which are also associated with risk of T2D, even more strongly than the common variant [98,99]. Among the 40 non-synonymous variants described by Bonnefond *et al.* [99], 24 very rare variants (MAF <0.1%) showed decreased receptor function: four led to total loss of function (p.Ala42Pro, p.Leu60Arg, p.Pro95Leu and p.Tyr308Ser), and 19 displayed impaired spontaneous or melatonin-induced activation of the G-proteins coupled to the receptor or reduced spontaneous beta-arrestin recruitment [98,99]. The variants with defects in melatonin-induced activation of the receptor were strongly associated with increased T2D risk (OR 3.25, 95% CI 1.73; 6.10,  $P = 2.4 \times 10^{-4}$ ). An interesting finding was that some variants also affected the spontaneous receptor activity, which was also associated with T2D risk. Notably, one of the variants showed a robust gain-of-phenotype. Unfortunately, no data on human physiology are available for these interesting variants. Without such data, the dispute on whether too much or too little melatonin signaling in the beta-cells predisposes to diabetes, is hard to solve [100,101]. Perhaps both views are true. Obviously the “crime-scene” does not even need to be in the islets, it could also be in other tissues, e.g. the CNS with high level of *MTNR1B* expression.

Another contradictory topic is, whether excess melatonin is good or bad for glucose-control. Melatonin can affect insulin secretion directly, but it can also affect glucose levels through non-beta-cell mechanisms - direct effects on glucose uptake, glucagon secretion, glycogen synthesis and inhibition of adrenocorticotrophic hormone (ACTH) secretion have been proposed [87,102].

Lower melatonin secretion was independently associated with a higher risk of developing type 2 diabetes in a nested case-control study within the Nurses' Health Study cohort [103]. Compared with women in the highest ratio category of urine 6-sulfatoxymelatonin to creatinine (reflecting melatonin secretion during the night), those in the lowest category had a multivariable odds ratio of 2.17 (95% CI, 1.18-3.98) of developing type 2 diabetes. Besides that study, human *in vivo* metabolic data are scarce, and melatonin treatment trials have included small numbers of patients suffering from various conditions that can themselves affect glucose control [102]. For example, if melatonin treatment in an anxiety disorder led to better glycaemic level, that could be due to melatonin affecting glycaemia (e.g. via insulin secretion) or due to reducing anxiety and thus increasing insulin sensitivity. Acute and long-term effects as well as effects of small or high-dose treatment can differ.

## Conclusions and perspectives

While modern techniques have revealed a multitude of genetic defects associated with diabetes, detailed human data on related beta-cell dysfunction are scarce and seldom analyzed in relation to the need of insulin, (insulin sensitivity). The genetic defects associated with beta-cell dysfunction can impact either structural (beta-cell mass) or functional (insulin secretion) fate of beta-cells – or both. Efforts to distinguish between these two dimensions are crucial in order to understand the pathogenesis of diabetes. Hence, beta-cell specific radioligands and imaging techniques that would allow for *in vivo* functional imaging of beta-cell mass and function are called for.

Human studies on type 2 diabetes often promote a simplified view on homogeneous beta-cells working in isolation. However, the approach neglects various physiological factors. Recent studies suggest operational heterogeneity among the beta-cells: a fraction of beta-cells in an islet resembles pacemakers as they orchestrate overall response to glucose [104]. Further, even in adults, beta-cells might lose cellular identity, and evidence is mounting on beta-cell de-differentiation and trans-differentiation [105]. Without the possibility to obtain biopsies, these

phenomena are practically impossible to study *in vivo*. Hopefully they can be addressed with studies involving biomarkers or liquid biopsies utilizing exosomal RNA from plasma or urine [106]. It is worth emphasizing, that beta-cells also interact with other cells in the endocrine and exocrine pancreas [107,108] as well as other organs – through hormonal and neural signals. A key hormone of glucose homeostasis, glucagon has attracted little attention compared to insulin, despite inappropriate hyperglucagonemia featuring in both type 1 and type 2 diabetes [109,110]. An interesting example is a variant in the *PCSK2* gene reported to decrease both insulin and glucagon secretion, which would have counteracting effects on glucose concentrations [111]. Finally, derangement of the pulsatile pattern of secretion of insulin and glucagon in diabetic individuals is an area not captured with the phenotyping efforts of today [112,113].

### **How should future studies be designed?**

It would be important to develop shared protocols between study groups for detailed phenotyping of carriers of the identified variants and carefully matched control groups. Such protocols should use different stimuli and suppressors of insulin and glucagon secretion together with dynamic functional imaging. Similarly to pipelines and algorithms for genetic curation of the variants, we call for standardized pipelines for phenotypic studies.

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## Figure and table legends

**TABLE 1.** A clinical assessment of the overall strength of the evidence for an association with an insulin secretion defect of selected genetic variants. Data are from the clustering analysis by Mahajan *et al.*[77], who found 29 variants associated with insulin secretion, eight were associated with high proinsulin (cluster 1) and 21 with low proinsulin (cluster 2), whereas another 35 variants were considered likely to have an impact on both insulin secretion and insulin action (cluster 3); (Supplementary figure 6B in [77]). We included the variants with data available for corrected insulin response adjusted for insulin sensitivity during an OGTT (CIR\*ISI), HOMA $\beta$ , 2-hour glucose and proinsulin, and either  $P < 1 \times 10^{-4}$  for any of the traits (shown in green), or nominally significant ( $P < 0.05$ ) association in case of CIR\*ISI or proinsulin (shown in yellow) (data from Supplementary table 11 in [77]). HOMA-IR  $P > 0.05$  for all associations except SPRY2 (0.0097), for ISI  $P > 0.05$  for all associations except for GIPR ( $> 0.02$ ) and SPRY2 (0.0036) (data not shown, see supplementary table 11 in [77]).

**FIGURE 1.** The relationship between HOMA $\beta$  (insulin secretion) and HOMA-IR (insulin sensitivity) in the PPP-Botnia Study [114] (of 3706 individuals, 3463 visualized within the display range), a population-based study in Western Finland. Closed black circles (●) represent individuals with normoglycemia (FPG  $\leq 5.5$  mmol/l) and good insulin sensitivity (defined as HOMA-IR  $\leq 1.0$ ), and open grey circles (○) represent other participants. Clearly, HOMA $\beta$  alone tells little about the insulin secretion capacity of the normoglycemic individuals as the values are scattered along the axis for HOMA $\beta$ , which highlights the importance to assess insulin secretion together with insulin sensitivity and glycaemic status.

**FIGURE 2.** Comparison of estimates of insulin sensitivity (panels A and B) and insulin secretion (panels C-F) based on samples taken at fasting (HOMA-IR, HOMA $\beta$ ) or during an OGTT (ISI<sub>30</sub>, CIR, 2-hour C-peptide) in individuals with (left panels, N=109-114) or without (right panels, N=3336-3439) diabetes from the population-based PPP-Botnia Study [114]. Diabetes was defined as FPG  $\geq 7$  mmol/l. Cases with extreme values were excluded (FGP  $\geq 10$  mmol/l, CIR  $< 0.25$  or  $>$

100, HOMA-IR > 16, ISI < 10, or 2-hour C-peptide > 5 nmol/l). HOMA-IR inversely correlates rather well with the OGTT-derived insulin sensitivity index (ISI, based on 0-, 30-, and 120-minute values) in both diabetic (panel A) and non-diabetic (panel B) individuals, whereas correlation of HOMA $\beta$  with either corrected insulin response at 30 minutes (CIR, panel C for diabetic and panel D for non-diabetic) or C-peptide response at 2 hours during an OGTT (panel E for diabetic and panel F for non-diabetic) is rather poor especially in non-diabetic individuals, underlining the biological difference between fasting and stimulated insulin secretion.

**FIGURE 3.** Age at diagnosis of diabetes differs largely among heterozygous carriers of the monogenic HNF1A variant (p.Gly292fs) from three big Finnish families (previously families B, C, D in [49]). Family members born in the later years were diagnosed earlier than those born earlier. After it had become apparent that the risk of diabetes was particularly high in these families, the finding is probably explained by an increased awareness and familial screening rather than an actual change in phenotype. The year 1975 was chosen as the cut-off to allow a sufficient number in both groups (~2/3 before and 1/3 after the year).

**FIGURE 4A.** Examples of heterogeneity in glucose and insulin responses during an OGTT in selected carriers heterozygous for the most common *HNF1A-MODY* variant p.Gly292fs. *Patient #1:* A 28-year-old lean woman has an OGTT response characteristic for HNF1A-MODY: fasting euglycemia precedes a steep post-challenge increase in plasma glucose, whereas the insulin response is relatively flat. *Patient #2:* a 33-year-old man with metabolic syndrome also shows a characteristic OGTT response for HNF1A-MODY (BMI 35 kg/m<sup>2</sup>, waist circumference 110 cm, serum triglycerides 3.73 mmol/l, HDL cholesterol 0.85 mmol/l, hypertensive blood pressure). *Patient #3:* a 29-year-old man has a good insulin response and surprisingly steady euglycemia throughout the OGTT despite the pathogenic *HNF1A* variant and obesity (BMI 33 kg/m<sup>2</sup>).

**FIGURE 4B.** Heterogeneous glucose and insulin responses during an OGTT in selected carriers of pathogenic *GCK* variants. *Patient #4:* A 19-year-old lean man shows an OGTT response

characteristic for GCK-MODY (p.Val181Ala): mild fasting hyperglycemia and low fasting insulin concentration followed by a mild rise in glucose, which triggers an insulin response at 30 minutes.

*Patient #5:* Some patients like this lean 46-year-old woman (#5) with a more drastic p.Gly261Arg variant [115–117] presented with marked post-challenge increase in glucose and weak insulin secretory response. *Patient #6:* A 45-year-old man with p.Met235Thr and metabolic syndrome (dyslipidemia, hypertension, waist circumference of 101 cm and BMI of 28 kg/m<sup>2</sup>) demonstrates a mixed OGTT response with features of both GCK-MODY and T2D.



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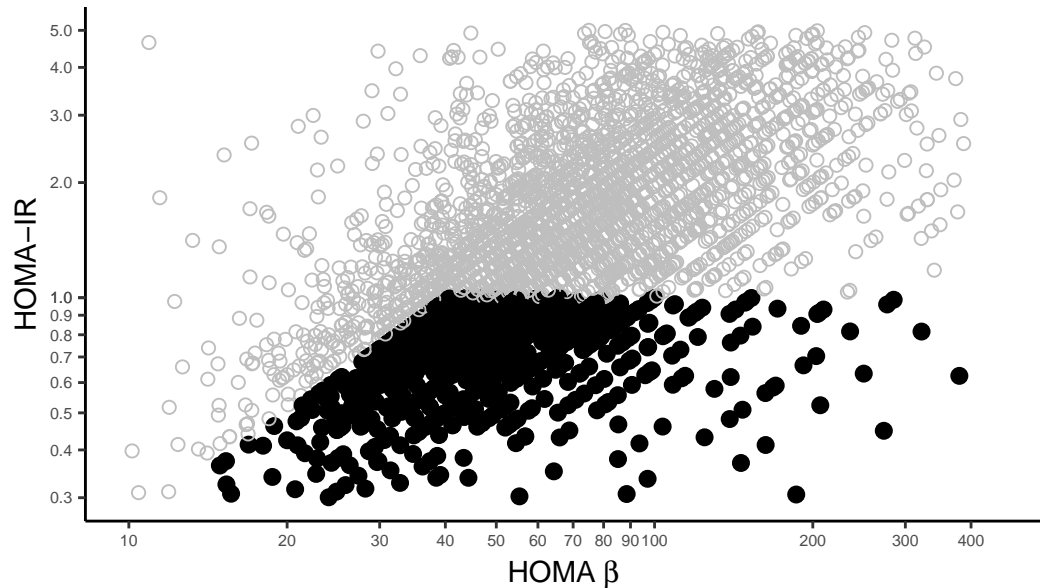
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Variant		Insulin secretion cluster	Overall evidence for insulin secretion defect			CIR*ISI N~5000			HOMAβ N~46000			2-hr glucose N~50000			Proinsulin N~10000		
						Beta	SE	P-value	Beta	SE	P-value	Beta	SE	P-value	Beta	SE	P-value
Low CIR (30 min insulin response)																	
MTNR1B	rs10830963	1	STRONG	-0.190	0.0250	4.73x10 <sup>-14</sup>	-0.0390	0.00400	8.60x10 <sup>-23</sup>	-0.00251	0.00968	0.796	-0.00400	0.00470	0.399		
CDKAL1	rs7756992	2	STRONG	-0.110	0.0230	8.13x10 <sup>-7</sup>	-0.00950	0.00360	0.00754	0.0422	0.00973	2.59x10 <sup>-5</sup>	-0.0110	0.00420	0.0107		
HHEX-IDE	rs5015480	2	STRONG	-0.100	0.0220	3.05x10 <sup>-6</sup>	-0.00470	0.00330	0.157	0.0372	0.00878	3.17x10 <sup>-5</sup>	0.00130	0.00390	0.733		
C2CD4A-B	rs4502156	2	STRONG	-0.100	0.0220	3.74x10 <sup>-6</sup>	-0.00990	0.00340	0.00357	-0.0475	0.00884	1.84x10 <sup>-8</sup>	-0.00170	0.00390	0.669		
CDKN2A-B	rs10965250	2	STRONG	-0.110	0.0300	0.000493	-0.00760	0.00450	0.0886	0.0328	0.0126	0.00868	0.00310	0.00510	0.545		
WSCD2	rs3764002	3	PROBABLE	-0.0950	0.0310	0.00194	0.00620	0.00420	0.138	-0.00490	0.00969	0.615	-0.00300	0.00510	0.560		
SLC30A8	rs13266634	1	STRONG	-0.0700	0.0240	0.00408	-0.0160	0.00380	2.36x10 <sup>-5</sup>	0.0165	0.00936	0.0991	-0.00360	0.00460	0.441		
TCF7L2	rs7903146	1	STRONG	-0.0660	0.0250	0.00876	-0.0200	0.00380	1.39x10 <sup>-7</sup>	0.0845	0.0102	1.58x10 <sup>-18</sup>	-0.0120	0.00430	0.0046		
BCAR1	rs7202877	3		-0.0830	0.0340	0.0154	-0.00340	0.00530	0.524	0.0374	0.0137	0.00431	-0.00600	0.00630	0.339		
ANK1	rs516946	2	STRONG	-0.0500	0.0250	0.0444	-0.0130	0.00380	0.000529	0.0414	0.0103	5.60x10 <sup>-5</sup>	-0.00930	0.00430	0.0318		
Low HOMAβ and high 2h-glucose																	
GCK	rs730497	1	STRONG	-0.0600	0.0330	0.0732	-0.0250	0.00460	3.84x10 <sup>-8</sup>	0.100	0.0124	9.21x10 <sup>-17</sup>	0.00570	0.00530	0.279		
ADCY5	rs11708067	1	STRONG	-0.0290	0.0290	0.319	-0.0160	0.00430	0.000177	0.0892	0.0107	4.26x10 <sup>-17</sup>	-0.00310	0.00490	0.530		
CENTD2	rs11603334	2	POSSIBLE	-0.0170	0.0280	0.550	-0.0160	0.00420	0.000138	0.0338	0.0114	0.00179	-0.0120	0.00510	0.0227		
GLIS3	rs10758593	2	POSSIBLE	-0,022	0.0210	0.308	-0.0150	0.00330	1.33x10 <sup>-5</sup>	0.0257	0.00887	0.00387	-0.00640	0.00390	0.0995		
High 2h-glucose																	
HNF1A	rs1169288	1	POSSIBLE	-0.0580	0.0230	0.0107	-0.00560	0.00350	0.111	0.0605	0.0108	7.86x10 <sup>-9</sup>	-0.00510	0.00410	0.211		
GIPR	rs8108269	3	POSSIBLE	-0.0450	0.0260	0.0806	-0.00260	0.00410	0.531	0.0389	0.0103	5.25x10 <sup>-5</sup>	-0.00220	0.00480	0.655		
ABO	rs505922	1	POSSIBLE	-0.0360	0.0220	0.105	-0.00170	0.00340	0.612	0.0491	0.00919	2.31x10 <sup>-8</sup>	0.00290	0.00400	0.474		
GIPR	rs1800437	3	POSSIBLE	-0.0420	0.0330	0.209	0.000800	0.00440	0.858	0.103	0.0108	2.59x10 <sup>-23</sup>	-0.00400	0.00520	0.446		
IGF2BP2	rs7633675	2	PROBABLE	-0.0270	0.0230	0.232	-0.0110	0.00350	0.00187	0.0499	0.0103	6.65x10 <sup>-7</sup>	-0.00520	0.00410	0.205		
MLX	rs665268	3	POSSIBLE	-0.0150	0.0250	0.556	0.00690	0.00370	0.0601	0.0422	0.0100	3.59x10 <sup>-6</sup>	0.00700	0.00430	0.101		
KCNQ1	rs2237892	2	POSSIBLE	-0.0200	0.0490	0.682	-0.0200	0.00730	0.00578	0.0607	0.0177	0.000187	-0.0190	0.00860	0.0305		
Low HOMAβ or proinsulin																	
DGKB	rs2191349	2	POSSIBLE	-0.0100	0.0200	0.609	-0.0210	0.00330	5.04x10 <sup>-11</sup>	0.00142	0.00879	0.791	-0.00500	0.00380	0.188		
PROX1	rs340874	2	POSSIBLE	-0.0310	0.0220	0.147	-0.0140	0.00340	3.43x10 <sup>-5</sup>	0.0233	0.00885	0.0166	-0.00850	0.00410	0.0365		
SPRY2	rs1359790	3		0.0230	0.0230	0.304	-0.00880	0.00360	0.0158	0.0109	0.00969	0.194	-0.0120	0.00420	0.00424		
ZZEF1	rs781831	3		0.0150	0.0210	0.482	-0.000954	0.00894	0.688	-0.00560	0.00330	0.0956	-0.00850	0.00390	0.0304		

**Figure 1**

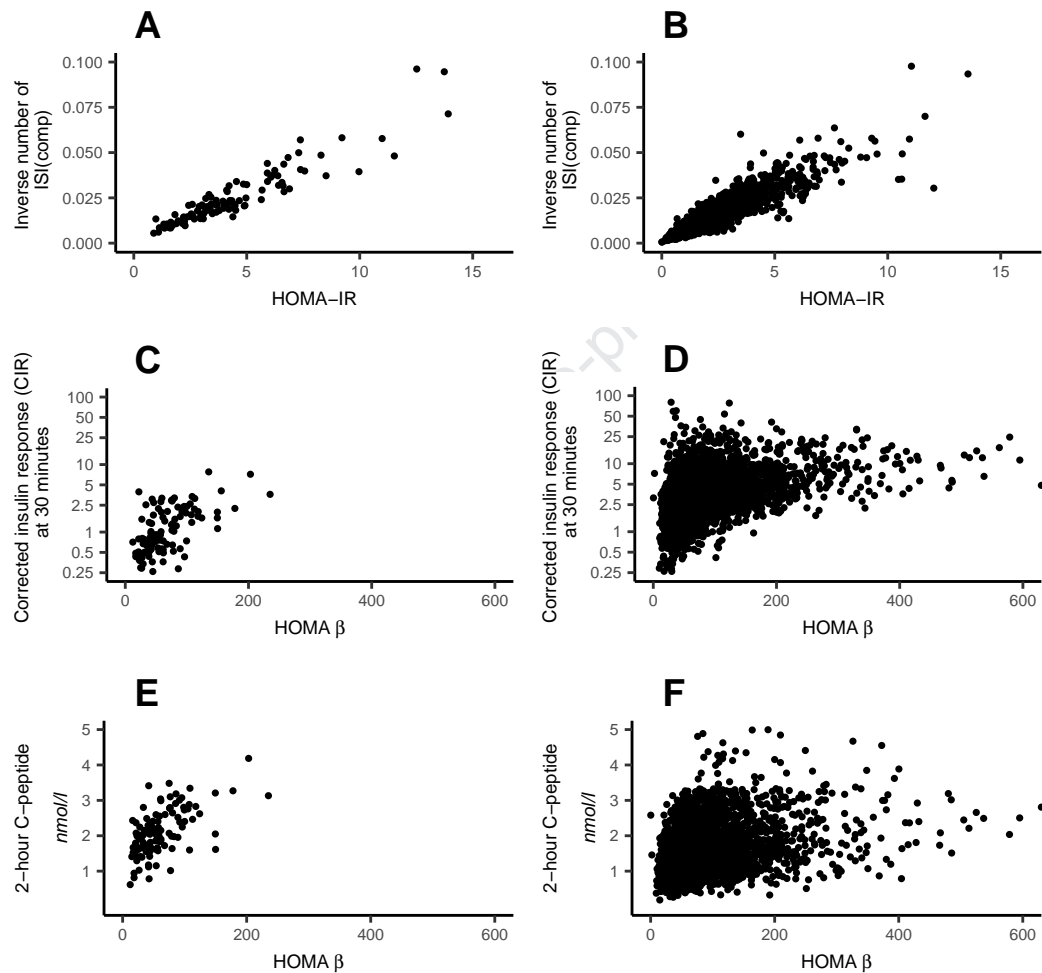
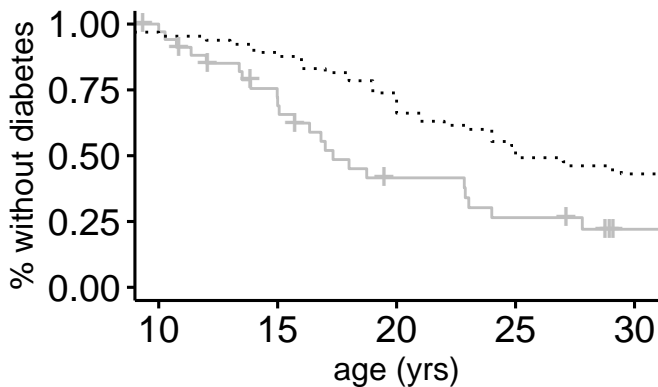


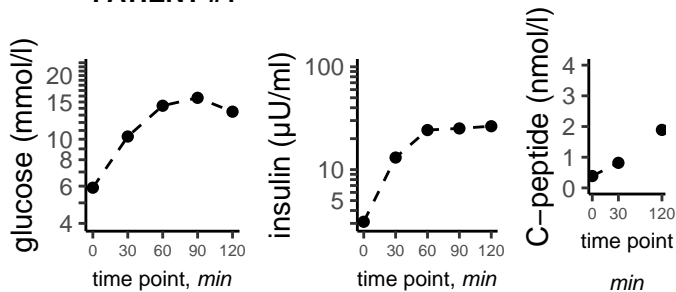
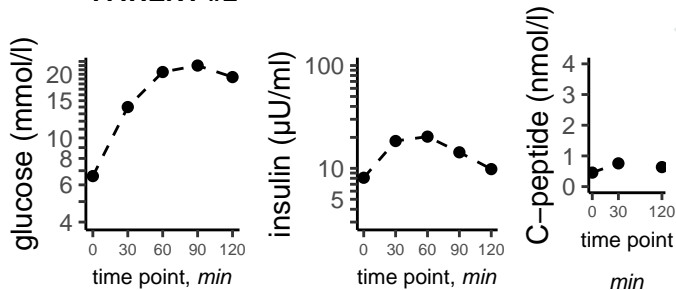
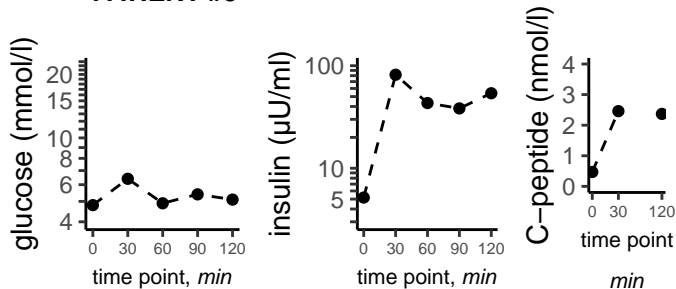
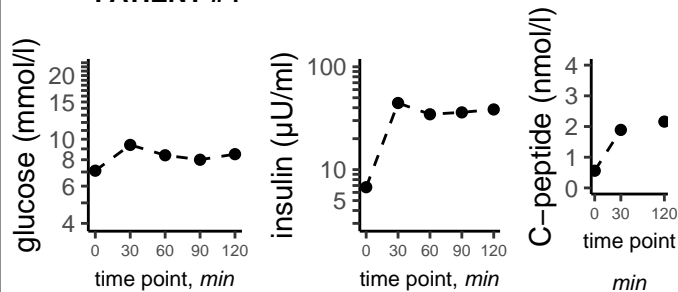
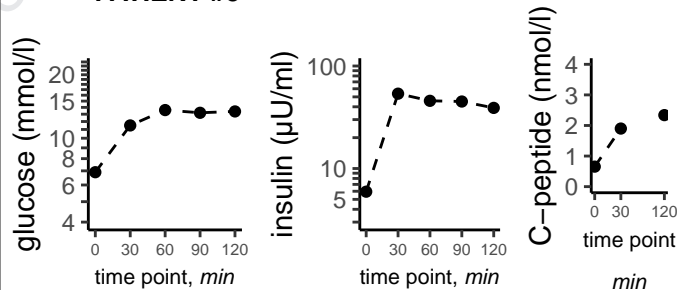
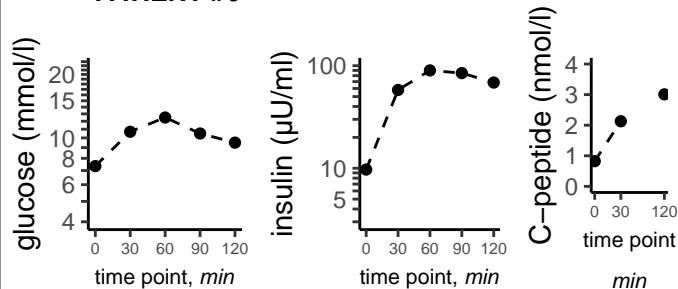
Figure 3



Number at risk

	34	22	11	7	2
	63	58	48	35	28

born in 1975 or later    born before 1975

**A) HNF1A-MODY****PATIENT #1****PATIENT #2****PATIENT #3****B) GCK-MODY****PATIENT #4****PATIENT #5****PATIENT #6**

Human physiology of the numerous genetic variants, associated with diabetes-related traits, has been poorly characterized.

Few variants can unequivocally be interpreted to be associated with defective insulin secretion across studies.

The different dysregulated processes, leading to beta-cell dysfunction, cannot be studied directly, so we need to rely on proxies and indices based on measuring blood samples.

Insulin secretion should be evaluated in parallel with insulin sensitivity

The available tests cannot distinguish between reduced beta-cell mass and defective beta-cell function.